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(54) Title: EQUINE HERPES VIRUS TEMPERATURE SENSITIVE MUTANT AND LIVE VACCINE THEREOF

(57) Abstract: The present invention relates to an equine abortion virus (EHV-1) mutant which is temperature sensitive at the body temperature of the host animal, more specifically at a temperature of 38.5 °C or higher. The temperature sensitive mutant can be used in vaccination to protect susceptible animals against EHV-1 infection. The invention furthermore relates to live vaccines derived from said mutant.

EQUINE HERPES VIRUS TEMPERATURE SENSITIVE MUTANT AND LIVE VACCINE THEREOF

The present invention relates to an equine abortion virus mutant, a process for the preparation of said mutant, use of said mutant and live vaccines derived from said mutant.

Equine abortion virus (EHV-1), a herpes virus, is a major equine pathogen responsible for viral-induced abortion, neurological disease such as paresis, infections of the upper respiratory tract, and neonatal foal disease (NFD). NFD results from close to term transplacental infection of fetuses, which are born weak with severe respiratory disease and some with jaundice due to liver infection by EHV-1. These animals usually die within a few days after birth. Equine rhinopneumonitis virus (EHV-4) is the major cause of acute respiratory tract disease ("rhinopneumonitis") and infects most horses during their first two years of life. Rhinopneumonitis is characterized by fever, anorexia, and profuse serous nasal discharge that later becomes mucopurulent. On rare occasions EHV4 infection causes abortion in pregnant mares. Furthermore EHV1 and EHV4 establish persistent, lifelong latent infections. Upon reactivation the viruses cause recurrent disease, accompanied by virus shedding and transmission to other animals.

Control of equine herpes virus infection and their diseases remain inadequate, in particular against EHV1 mediated abortions, paresis and neonatal foal disease resulting from close to term transplacental infection of foetus. Although inactivated as well as modified live vaccines are available, neither vaccine appears to block infection sufficiently, nor do they prevent the establishment of latency by wild-type virus. Hence there is a great need for safe vaccines with improved protection against field infections of these viruses, particularly against infections caused by EHV1.

The present invention provides for such vaccines.

In a first aspect the present invention provides for an EHV-1 Ts mutant as deposited at the European Collection of Animal Cell Culture (ECACC), Salisbury, Wiltshire SP4 0JG, UK on 10 June 1999 under accession number V99061001, and progeny thereof.

The EHV-1 Ts mutants according to the invention are furthermore phenotypically characterized in that

•they form small plaques when grown on several horse cell lines,

• they have lost their ability to grow on rabbit kidney cells, in particular RK13 cells, and

•they are limited in their ability to cause viraemia (that is, they are able to ;

The EHV-1 Ts mutants according to the invention have the advantage that replication is restricted to the upper respiratory tract of conventional equidae with no or limited ensuing viraemia. The Ts mutants are safe for pregnant mares while giving rise to significant immune stimulation following growth in the upper respiratory tract. The Ts mutants are not readily back-passaged form animal to animal thus limited in their potential for transmission and reversion.

For the purpose of this invention "progeny" is defined to include also all strains obtained by further serial passage of the deposited EHV-1 Ts mutant.

For the purpose of this invention, a temperature sensitive mutant is defined as a mutant virus which has an impaired growth at or above a certain temperature at which the wild type has a normal growth. The EHV-1 Ts mutants according to the present invention are characterized in that they are temperature sensitive at the body temperature of the host animal. The EHV-1 Ts mutants of the present invention do not replicate above a temperature of 38.5 to 39.0°C. Preferably the EHV-1 Ts mutants according to the invention do not replicate at a temperature of 38.5°C.

For the purpose of this invention, small plaques are defined as plaques that are at least half to one third the size of the plaques formed by the wild-type parent strain in equine cells.

For the purpose of this invention the "limited abilty to cause viraemia" is defined as the ability to cause no or low grade (that is, just detectable) vireamia for 1 to 3 or 4 days in some animals with respect to the ability of the parent strain to cause viraemia.

Temperature sensitive EHV-1 mutants according to the invention can be obtained by treatment of infected bovine, equine or other permissive cell cultures at 34°C with non-toxic concentrations of a mutagens such as 5-bromo-2-deoxy uridine, azacytidine and the like during viral replication in vitro, followed by biological cloning of progeny virus from said treated cultures in bovine or equine or other permissive cell lines.

The favorable properties of the Ts-mutants according to the invention makes them very suitable for use in the preparation of a vaccine. Thus, in a second aspect the present invention provides for a composition, in particular a vaccine composition, comprising an EHV-1 Ts-mutant according to the invention, and a pharmaceutically acceptable carrier or vehicle. More specifically, a (vaccine) composition according to the invention comprises the EHV1 Ts-mutant deposited at the ECACC, Salisbury, UK having accession number V99061001 and/or progeny thereof. Pharmaceutical acceptable carriers or vehicles that are suitable for use in a vaccine according to the invention are sterile water, saline, aqueous buffers such as PBS and the like. In addition a vaccine according to the invention may comprise other additives such as adjuvants, stabilizers, anti-oxidants and others.

The vaccine compositions according to the invention are safe and can be used to protect the equidae clinically and virologically against infections with EHV-1 and to protect against virus-induced abortions and paresis. In addition the vaccine according to the invention was found to stop trans-placental infection, thus protecting the newborn foal from the effects of neonatal foal disease. The vaccine composition according to the present invention can be administered not only to horses but also to other animals that are susceptible to EHV-1 infection such as donkeys, zebra's and the like. Cattle which have been reported to be susceptible to EHV-1 and EHV-4 infection can also be treated with the vaccine according to the invention.

It was furthermore surprisingly found that vaccines comprising an EHV-1 Ts-mutant according to the invention not only protect against EHV-1 infections but also against the disease and the associated virus shedding following EHV-4 infection. Thus such a vaccine can be useful to obtain cross-protection in the vaccinated equidae. Said vaccines give rise to improved protection thus effectively blocking infection with wild-type viruses.

Vaccine compositions according to the invention can be prepared following standard procedures. A vaccine according to the invention preferably is a live vaccine. For the preparation of the live vaccine, the seed virus of the EHV-Ts mutant can be grown on a cell culture, such as primary or secondary bovine kidney or equine cells. The viruses thus grown can be harvested by collecting the tissue cell culture fluids and/or cells. Optionally, during harvesting the yield of the viruses can be promoted by techniques that improve the liberation of the infective particles from the growth substrate, e.g. sonication. The live vaccine may be prepared in the form

of a suspension or may be lyophilized.

Pharmaceutical acceptable carriers that are suitable for use in a vaccine according to the invention are sterile water, saline, aqueous buffers such as PBS and the like. In addition a vaccine according to the invention may comprise other additives such as adjuvants, stabilizers, anti-oxidants and others.

Suitable stabilizers are for example carbohydrates including sorbitol, mannitol, starch, sucrose, dextran and glucose, proteins and degradation products thereof including but not limited to albumin and casein, protein-containing agents such as bovine serum or skimmed milk, and buffers including but not limited to alkali metal phosphates. In lyophilized vaccine compositions it is preferable to add one or more stabilizers.

Suitable adjuvants include but are not limited to aluminum hydroxide, phosphate or oxide, amphigen, tocopherols, monophosphenyl lipid A, muramyl dipeptide, oil emulsions, glucans, carbomers, block-copolymers, cytokines and saponins such as Quil A. The amount of adjuvant added depends on the nature of the adjuvant itself.

EHV-1 Ts mutants according to the invention are preferably administered to conventional, seronegative animals varying in ages from a few days to several years, including those in-foal. The vaccine can be administered to the animals via non-parenterally administration routes, including but not limited to intradermal, oral, spraying, aerosol, intra-ocular, and intranasal administration. Alternatively, the vaccine can be administered via parenteral administration routes. Preferably the vaccine is administered intradermally or intranasally.

In general the EHV-1 Ts mutant virus is administered in an amount that is effective to induce protection against EHV-1 infection. The dose generally will depend on the route of administration, the time of administration, as well as age, health and diet of the animal to be vaccinated. The virus can be administered in an amount between 10^2 and 10^9 pfu/dose per animal, preferably between 10^3 and 10^5 pfu/dose and more preferably at 10^4 pfu/dose per animal.

The vaccines according to the invention also may be given simultaneously or concomitantly with other live or inactivated vaccines. These additional vaccines can be administered non-parenterally or parenterally. Preferably the additional vaccines are recommended for parenteral administration.

Claims

1. A temperature-sensitive (Ts) mutant of Equine abortion virus (EHV-1) characterized in that the virus is the EHV1 Ts-mutant deposited at the ECACC under Accession No. V99061001, or progeny thereof.

2. Pharmaceutical composition comprising a temperature-sensitive mutant according to claim 1 and a pharmaceutical acceptable vehicle or carrier.

3. Vaccine for the prevention and/or treatment of EHV-1 infections in equidae comprising a temperature-sensitive mutant virus according to claim 1 and a pharmaceutically acceptable carrier or diluent.

4. Use of a temperature sensitive mutant according to claim 1 in the manufacture of a vaccine for the prevention and/or treatment of EHV-1 infections.

5. A method for the immunization of an animal against EHV-1 infection comprising administering to said animal a vaccine according to claim 4.